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DIFFERENCES IN PURITY OF COMMERICAL SAMPLES OF HEMICHOLINIUM-3:--ETC(U)

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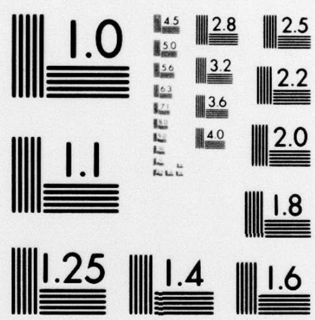
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DIFFERENCES IN PURITY OF COMMERCIAL SAMPLES OF HEMICHOLINIUM-3: EFFECTS ON HIGH AFFINITY CHOLINE UPTAKE, NEUROMUSCULAR TRANSMISSION, ACETYLCHOLINESTERASE AND CARBACHOL AND ACETYLCHOLINE CONTRACTIONS

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Summary

Commercial samples of hemicholinium-3 (HC-3) have been found to vary in colour (from white to a sandy-yellow colour) and chemical composition. There were no significant differences between the various HC-3 samples with regards to inhibition of high affinity choline uptake into synaptosomes or inhibition of neuromuscular transmission in the chick biventer cervicis (CBC) preparation. Yellow HC-3 inhibited acetylcholinesterase more than white HC-3 with I_{50} of 4.8×10^{-5} M and 3.3×10^{-4} M, respectively. Carbachol-induced contractions of the CBC preparation were inhibited more by yellow HC-3 than white HC-3; the opposite was true for acetylcholine-induced contractions. The results indicated that there is a minor contaminant in yellow HC-3 other than deanol which was a potent inhibitor of acetylcholinesterase and the carbachol response.

Hemicholinium-3 (HC-3), first described by Long and Schueler (1), is a potent inhibitor of the high affinity choline uptake system in the presynaptic neuron (2). Blockade of the high affinity choline uptake system leads to impairment of acetylcholine (ACh) synthesis and release eventually resulting in blockade of synaptic transmission. HC-3 samples from commercial sources were found to vary in colour and texture from a white crystalline to a sandy yellow granular material. The purpose of this study was to determine if there was a difference in the pharmacological properties of HC-3 obtained from the various commercial sources on high affinity choline uptake into synaptosomes, neuromuscular (NM) transmission in the chick biventer cervicis (CBC) preparation, anti-acetylcholinesterase (AChE) activity and ACh- and carbachol-induced contractions of the CBC preparation.

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Methods

Anti-AChE activity was measured by the titrimetric procedure (3) using purified bovine erythrocyte AChE (Sigma). At pH 7.4 HC-3 was added one minute before the substrate ACh iodide. The CBC preparation according to Ginsborg and Warriner (4) was used for studies on NM transmission and the effect of HC-3 on agonist-induced contractions. Chicks were 6 to 15 days old. The CBC muscle was maintained at 37°C in a water-jacketed organ-bath containing Krebs-Henseleit solution of the following composition (mM); NaCl, 118; KCl, 4.72; $MgSO_4$, 1.18; CaCl, 2.52; KH_2PO_4, 1.18; $NaHCO_3$, 25.0; and dextrose, 11.1 and gassed with oxygen containing 5% CO_2. The preparation was stimulated for 60 min and HC-3 was added at "0" time. Contractions were recorded using an isometric muscle transducer connected to an ink-writting dynograph. The preparation of synaptosomes and conditions of the choline uptake experiments were as described by Rylett and Colhoun (5).

HC-3 samples used were as follows: Eastman Kodak (Lot #B3C); ICN (Lot #2804-1A); and Aldrich Chemical Co. (Lot #082361, #122171, #090591, #072557, and #041877). All the commercial samples were stated by the manufacturer to be 95 - 99% pure HC-3. Some HC-3 samples (Eastman Kodak, lot #B3C; ICN, lot #2804-1A; Aldrich Chemical Co., #072557 and #041877) were obtained directly from the manufacturers by the authors and the other HC-3 samples used were kindly supplied by the following individuals from their own reagent inventory (Dr. C.C. Chang, Aldrich #082361; Dr. B. Collier, Aldrich #122171 and VBH5-83-1; Dr. J.C. Szerb, Aldrich #041877; Dr. D.J. Jenden, sample of recrystallized Eastman HC-3 (6); Dr. J.G. Clement, Aldrich #090591). All drug concentrations refer to final concentrations.

Results

Samples of HC-3 varied in colour and texture from a white-crystalline to a sandy-yellow granular material. Early Aldrich products (Lot #082361, #122171 and #090591) were white crystalline material. However, the two latest batch numbers (Lot #072557 and #041877) are sandy-yellow in colour, similar to the samples obtained from ICN and Eastman Kodak. The HC-3 samples were examined by thin layer chromatography using Woelm Silica Gel plates (hardened, organic binder) with methanol-acetic acid 9:1 as the eluant. The plates were visualized with short-wave UV light (254 nm) and with iodine vapor. The yellow coloured, commercially obtained samples showed numerous impurities with HC-3 itself running at R_f 0.41. The most significant impurities, those which fluoresced under UV light (running between R_f 0.2 and the origin), were absent or markedly reduced in recrystallized or white HC-3 samples.

The effect of the various samples of HC-3 on inhibition of high affinity choline uptake into synaptosomes was investigated. The results in Table I indicate that there was little variation between the samples of HC-3 with regards to NM inhibition of high affinity choline uptake. The contamination present in yellow HC-3 samples, as noted from the TLC results, did not significantly affect high affinity choline uptake into synaptosomes.

The results in Figure 1 demonstrate that the extent and onset of the presynaptic NM inhibition in the CBC preparation produced by yellow and white HC-3 was not significantly different.

TABLE I

Inhibition of High Affinity Choline Uptake into Synaptosomes by Various Samples of HC-3

Sample	% Inhibition	
	range	mean
VBH5-83-1	79.2 - 84.7	81.9*
Jenden's	77.2 - 84.3	80.8
Eastman #B3C	76.9 - 81.1	80.0
Aldrich #082361	77.8 - 80.0	78.9
Aldrich #122171	72.1 - 78.9	75.5
Aldrich #072557	77.4 - 81.9	79.6
Aldrich #041877	79.2 - 83.0	81.1
ICN #2804-IA	77.7 - 84.7	81.2

* mean of two separate experiments utilizing duplicate samples. HC-3 was added to the synaptosomal suspension to give a final concentration of 0.1 μM and incubated for 10 min prior to the addition of choline. ^{3}H-choline, (1 μM; 1.82 mCi/mmole) was added and samples were incubated for an additional 7 min during which high affinity choline transport was linear.

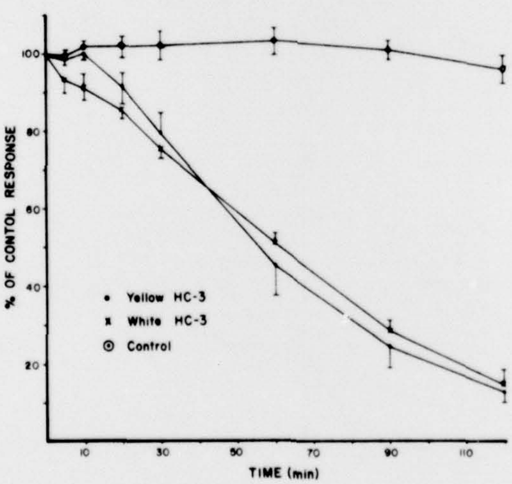

FIG. 1

Presynaptic-type NM inhibition produced by White and Yellow HC-3 in the CBC Preparation
[yellow HC-3] = 2.9 x 10^{-6} M, Aldrich #041877;
[white HC-3] = 2.9 x 10^{-6} M, Aldrich #090591.
The frequency of stimulation was 1.0 Hz. Each point represents the mean ± SEM of at least four separate observations.

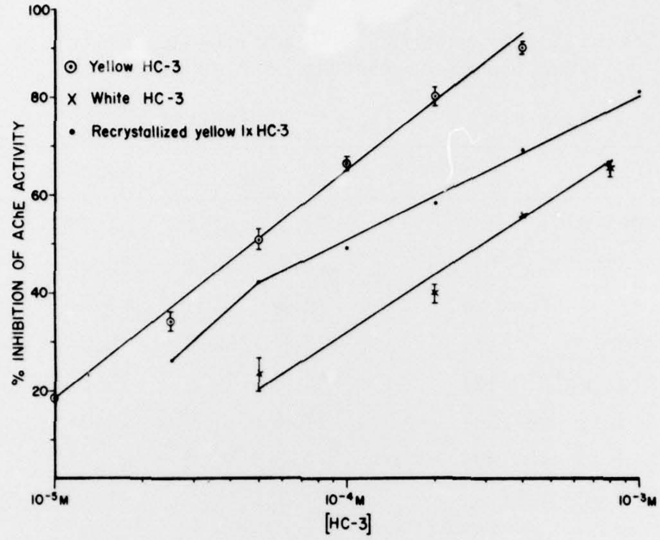

FIG. 2

In vitro Inhibition of Purified Bovine Erythrocyte AChE by HC-3 yellow HC-3 samples (ICN #2804-1A; Eastman #B3C; Aldrich #072557 and #041877) and white HC-3 samples (Aldrich #082361, #122171 and #090591; VBH5831; and Jenden's recrystallized HC-3) There were no significant differences among the various yellow and white samples with regard to inhibition of AChE, therefore the data were grouped according to colour and presented as above. Each point represents mean ± SEM of 12 - 15 observations.

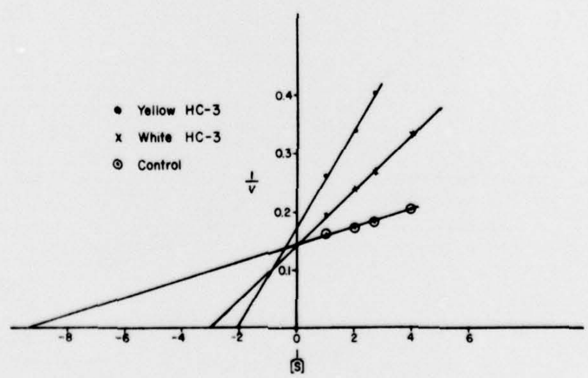

FIG. 3

Lineweaver-Burke Plot of the Inhibition of Purified Bovine Erythrocyte AChE by White and Yellow HC-3 (1×10^{-4}M). White HC-3 (Aldrich #090591) and yellow HC-3 (Aldrich #041877) were used. The temperature was 37°C and concentration of AChE was 0.01 unit/ml. These curves were constructed using the Ellman procedure as described by French et al. (7).

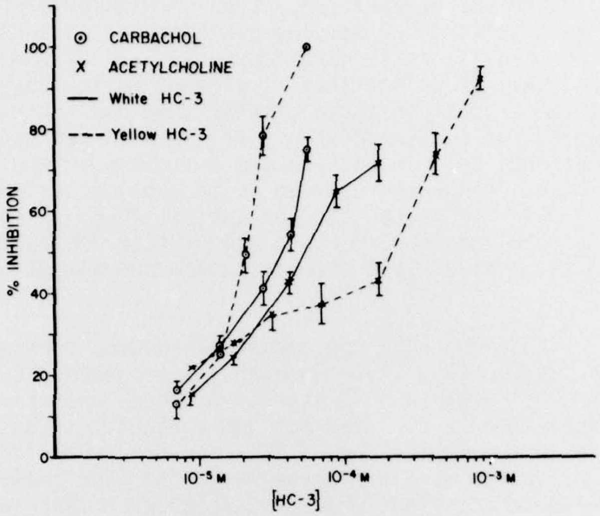

FIG. 4

Effect of Various Concentrations of HC-3 on the Agonist-induced Contractions of the CBC Preparation Produced by Equipotent Concentrations of ACh and Carbachol.
ACh or carbachol contractions in presence of either yellow HC-3 (#072557) or white HC-3 (#090591). Each point represents mean ± SEM of 8 - 12 observations. The agonist-induced contractions of the CBC preparation in the presence of HC-3 were expressed as a percent of the untreated control contraction. HC-3 was present for 1.0 min before and during addition of the agonist. Concentrations of carbachol and ACh were 4×10^{-5} M and 1.3×10^{-3} M, respectively which produced a contraction approximately 70% of maximum.

The anti-AChE activity of the samples of HC-3 was investigated and it was found that the yellow coloured HC-3 was a more potent inhibitor of AChE than white HC-3 with an I_{50} of 4.8×10^{-5} M and 3.3×10^{-4} M, respectively (Fig. 2). Also, the white HC-3 was a competitive inhibitor of AChE (Fig. 3), whereas the yellow HC-3 was a mixed-type inhibitor. Deanol (5×10^{-4} M), a precursor and a possible contaminant (6) of HC-3 did not inhibit AChE.

The effect of various concentrations of yellow and white HC-3 on carbachol and ACh-induced contractions of the CBC preparation was investigated. The results in Figure 4 show that larger concentrations of the yellow HC-3 were required to get the same degree of inhibition of ACh-induced contractions of the CBC preparation as the inhibition produced by white HC-3. In contrast, yellow HC-3 was a more potent inhibitor of carbachol-induced contractions than white HC-3.

Discussion

The results of this study demonstrate that even though the various commercial HC-3 preparations vary in colour and chemical make-up, this minor contamination does not affect significantly either the effect of

HC-3 on high affinity choline uptake into synaptosomes or the inhibition of NM transmission in the CBC preparation. Studies with bovine erythrocyte AChE indicate that yellow HC-3 samples contained an anti-AChE which must be highly potent (Fig. 1) as it comprises only 2.5% of sample weight according to manufacturers specifications of product purity however, it is capable of shifting the inhibition curve greater than one logarithmic unit to the left. Inhibition of AChE produced by yellow HC-3 recrystallized one time from boiling methanol fell in between the white and yellow commercial samples (Fig. 1). Deanol has been reported to be a major contaminant in HC-3 (6), however, 5×10^{-4} M deanol did not inhibit AChE. As previously found by Domino et al. (8) white HC-3 was a competitive inhibitor of AChE whereas yellow HC-3 was a mixed-type (competitive - non-competitive) inhibitor of AChE.

White HC-3 inhibited ACh- and carbachol-induced contractions of the CBC to a similar degree (Fig. 4). However, at a concentration where AChE inhibition produced by white HC-3 starts to become appreciable, a divergence of the inhibition curves for ACh and carbachol is noted (Fig. 4). The large difference in the inhibition of ACh- and carbachol-induced contractions produced by yellow HC-3 was probably due to AChE inhibition. ACh- and carbachol-induced contractions were inhibited to the same degree by d-tubocurarine (unpublished observations) suggesting that a post-synaptic blocking agent with negligible anti-AChE activity inhibits ACh and carbachol contractions to a similar degree.

Work is in progress to attempt to characterize the potent anti-AChE shown to be present in yellow coloured commercial samples of HC-3.

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